

# EPINEPHRINE INHIBITS THE ACTIVITY OF PHOSPHATIDATE PHOSPHOHYDROLASE OF ISOLATED HUMAN HEPATOCYTES

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## ABSTRACT

The effect of epinephrine on phosphatidate phosphohydrolase (PAP) activity of isolated human hepatocytes was studied. Epinephrine inhibited the enzyme activity progressively at concentrations above 0.1  $\mu\text{M}$ , reaching a maximum inhibition of 64.5% at 100  $\mu\text{M}$  concentration. Inclusion of alprenolol, a  $\beta$ -receptor blocker, in the incubation mixture abolished the inhibitory effect of epinephrine on PAP, whereas the  $\alpha$ -receptor antagonist phentolamine, or agonist phenylephrine, did not significantly change the hormone's effect. Addition of dibutyryl-cAMP or aminophylline (a cAMP phosphodiesterase inhibitor) to the incubation mixture together with epinephrine caused further enzyme inhibition reaching 65.6% and 63.7%, respectively, compared to 49% inhibition caused by epinephrine alone under the same conditions. Dibutyryl-cAMP alone also inhibited PAP activity (51%). The results suggested that epinephrine affects human hepatocyte PAP activity through  $\beta$ -adrenoceptor activation and cAMP is involved in the mechanism by which PAP activity is altered.

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## INTRODUCTION

Phosphatidate phosphohydrolase (PAP, EC. 3. 1. 3. 4.) that catalyzes the dephosphorylation of phosphatidate is believed to be a regulatory enzyme in triacylglycerol biosynthesis.<sup>1,2</sup> The activity of this enzyme is regulated by various mechanisms. It has been proposed that PAP in the cytosol is in its inactive form and that it becomes active when it is translocated to the membrane where glycerolipid synthesis occurs.<sup>3,4</sup> *In vitro* studies have shown that fatty acids promote this translocation with a concomitant increase in PAP activity.<sup>5</sup> The activity of PAP in rats was also affected by several hormones such as glucocorticoids,<sup>6,7</sup> thyroxine,<sup>8</sup> growth hormone,<sup>9</sup> glucagon and sex hormones.<sup>10</sup>

Our previous reports revealed that epinephrine decreased PAP activity when it was incubated with rat hepatocytes for a short period of time,<sup>11</sup> but exerted an opposite effect at

higher incubation periods.<sup>12</sup> *In vivo* administration of epinephrine to adrenalectomized rats also increased hepatic PAP activity in rats.<sup>13</sup> The mechanism by which epinephrine affects hepatic PAP activity, however, is still unknown. Also, very little information exists concerning the possible hormonal regulation of PAP in humans. In the present work the effect of epinephrine on PAP activity of human hepatocytes was examined and the receptors through which this hormone exerted its effect were identified.

## MATERIALS AND METHODS

### Chemicals

Phosphatidic acid (sodium salt), epinephrine, phenylephrine, dibutyryl-cAMP, alprenolol, phentolamine and aminophylline were obtained from Sigma Co.(USA). All other chemicals were of reagent grade.

### Human liver samples

Postmortem liver samples were removed by immediate autopsy after death caused by accident in hospitals affiliated to Isfahan University of Medical Sciences. The samples (15-20g) were washed with normal saline 3 times and kept in the same solution for hepatocyte preparation.

### Hepatocyte isolation and incubation

Hepatocytes were isolated in Krebs-Hansleit bicarbonate buffer as previously described.<sup>11</sup> Cell viability was assessed with trypan blue staining which generally exceeded 90%. The cells were incubated at 37°C in the above buffer under an atmosphere of 95% O<sub>2</sub> and 5% CO<sub>2</sub> (vol/vol) for 30 minutes with shaking (90 cycle/min). The incubation mixture contained 6×10<sup>6</sup> cells/mL with the indicated amounts of epinephrine or other agents in a total volume of 5 mL. When antagonists were present, a 15-minute preincubation was done before the addition of epinephrine.<sup>14</sup> The preincubation in the presence of aminophylline was 2-3 minutes.<sup>15</sup> After the incubation time, the cells were separated from the medium by centrifugation at 150 g for 1 min. The cells were washed 3 times with normal saline, resuspended in 3-volumes of 0.05 M Tris-HCl buffer, pH 7.5, containing 225 mM sucrose and 1 mM EDTA. The cells were homogenized for 8 min. using a Teflon homogenizer. The homogenate was centrifuged at 12000 g for 30 min. and the supernatant fluid was kept for the enzyme assay.

### Determination of PAP activity

The enzyme activity was measured by determining the release of inorganic phosphate from an aqueous dispersion of phosphatidate used as substrate as described before.<sup>16</sup> Each assay contained, in a volume of 0.5 mL: 50 mM Tris-HCl pH 7.4, 2 mM sodium phosphatidate, 1 mM dithiothreitol and an appropriate amount of the enzyme solution. Reactions were started by adding phosphatidate emulsion and after 10 min of incubation at 37°C, 1 mL of 10% trichloroacetic acid was added to stop the reaction, hence the concentration of Pi was determined.<sup>21</sup> One unit (U) of the enzyme is defined as the amount which catalyzes the release of 1μmole inorganic phosphate (Pi) per min. under standard assay conditions.

### Protein determination

Protein concentration was determined by the method of Lowry et al.<sup>17</sup>

## RESULTS

The hepatocytes were incubated with epinephrine at concentrations ranging from 0.1 μM to 100 μM. The results shown in Fig. 1 demonstrated that epinephrine inhibited

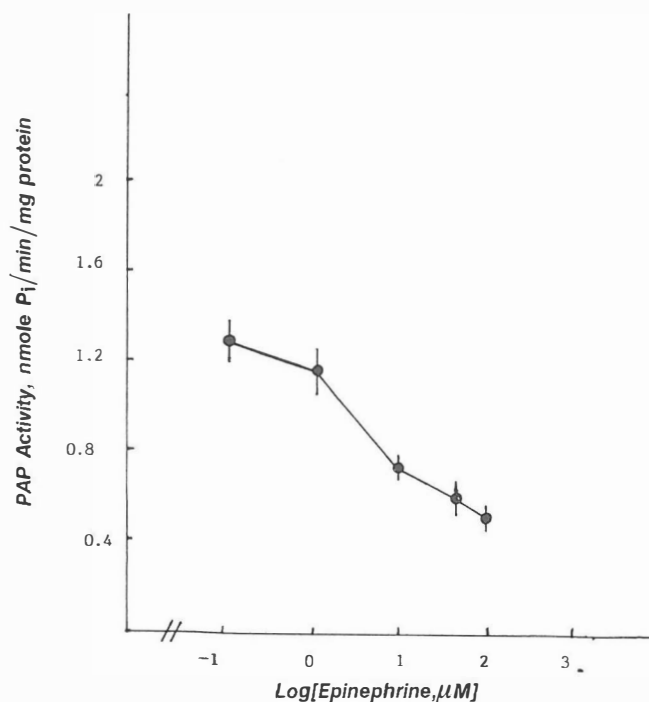


Fig. 1. Concentration-dependence of the effect of epinephrine on PAP activity of human hepatocytes. The cells (6×10<sup>6</sup>/mL) were incubated with epinephrine for 30 min at 37°C. The enzyme activity was measured as described in *Methods*. Values represent mean ± S.E. of 3 separate cell preparations.

PAP activity progressively up to a concentration of 100 μM at which the inhibition was 64%. The involvement of β-adrenoceptors in the mechanism by which epinephrine inhibited PAP activity is demonstrated in Table I. Inclusion of alprenolol (10 μM), a β-receptor blocker, in the incubation mixture together with epinephrine abolished the inhibitory effect of the hormone. Phentolamine (10 μM), an α-receptor antagonist, however, did not prevent the effect of epinephrine. Also, PAP activity inhibition by epinephrine did not significantly increase in the presence of phenylephrine (10 μM), an α-receptor agonist. Table I also shows that addition of dibutyryl-cAMP or aminophylline (a cAMP phosphodiesterase inhibitor) to the incubation mixture together with epinephrine caused further enzyme inhibition reaching 71.3% and 63.7%, respectively. Dibutyryl-cAMP alone also inhibited PAP activity by 51.5%. None of the reagents tested affected PAP activity when added to the assay mixture.

## DISCUSSION

The key step in regulation of glycerolipid synthesis is catalyzed by PAP. Several studies have examined the hormonal regulation of PAP in different mammalian tissues.<sup>7-13</sup> The results presented in this study demonstrate the short term inhibitory effect of epinephrine on PAP activity of

**Table I. The effect of epinephrine on PAP activity of isolated human hepatocytes in the presence and absence of  $\alpha$ - and  $\beta$ -receptor agonists and antagonists.**

Substance Added	PAP Activity	
	nmole Pi/min/mg Protein	Remaining %
None (control)	1.57 $\pm$ 0.14	100
Epinephrine (10 $\mu$ M)	0.80 $\pm$ 0.08*	51
Epinephrine (10 $\mu$ M)+alprenolol (10 $\mu$ M)	1.52 $\pm$ 0.11	96.8
Epinephrine (10 $\mu$ M)+phentolamine (10 $\mu$ M)	0.77 $\pm$ 0.06*	49
Epinephrine (10 $\mu$ M)+phenylephrine (10 $\mu$ M)	0.76 $\pm$ 0.09*	48.4
Epinephrine (10 $\mu$ M)+aminophylline (1 $\mu$ M)	0.57 $\pm$ 0.07*	36.3
Epinephrine (10 $\mu$ M)+dibutyryl-cAMP (10 $\mu$ M)	0.54 $\pm$ 0.09*	34.4
Dibutyryl-cAMP (10 $\mu$ M)	0.76 $\pm$ 0.05*	48.4

The cells ( $6 \times 10^6$  cells/mL) were incubated with the indicated agents for 30 min. at 37°C and PAP activity measured as described in the *Methods* section. Preincubation was 15 min for antagonists and 2 min for aminophylline. Values are mean  $\pm$  S.E. of 3 separate call preparations.

\* Statistically different from control ( $p < 0.05$ ).

isolated human hepatocytes.

The finding that alprenolol—but not phentolamine—abolished the inhibitory effect of epinephrine on PAP activity provided evidence that the hormone affects cell metabolism through  $\beta$ -adrenoceptor activation. Since the cAMP phosphodiesterase inhibitor aminophylline stimulated the hormone's effect and dibutyryl-cAMP alone also inhibited PAP activity, cAMP may be involved in the mechanism by which the activity of PAP is inhibited.

Other studies have shown that short term incubation of adipocytes with norepinephrine or palmitate elevates PAP activity and insulin antagonizes only the effect of norepinephrine.<sup>18</sup> It appears that in adipose tissue the increased cAMP brought about by the lipolytic effect of the hormone raises the intracellular concentration of fatty acids which in turn provides substrate for PAP. Fatty acids also activate PAP through translocation of the enzyme from the cytosol to microsomal membranes where it is highly active.<sup>5</sup> In the hepatocyte, however, cAMP inhibits fatty acid synthesis and stimulates their oxidation to CO<sub>2</sub> and ketone bodies.<sup>19</sup> This explains the inhibitory effect of epinephrine on human PAP activity mediated by cAMP. Another possibility is a probable phosphorylation/dephosphorylation of PAP in which cAMP is involved. In rats, cAMP in long term stimulates PAP synthesis but in short term causes enzyme translocation to the cytosol where it is metabolically inactive. Polyamines, however, exert an opposite effect.<sup>7,20</sup>

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